Review: A Flavor of Vanilla

Aroma, taste and mouthfeel

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Vanilla bean curing is a traditional process that has been carried out for hundreds of years, originating with the Totonac tribe of Mexico in the early 16th century.[1] A major stimulus to its global proliferation was the development of hand pollination and vegetative propagation in the 1830s.[1] The process is fairly simple, comprising five stages—blanching (or killing), sweating (or fermentation), sun drying/night storage, rack drying and conditioning.[2]

The Biochemistry and Chemistry of Curing

The curing process is a complex and multifaceted sequence of interventions that takes place over a period of about 150 days, turning an unflavored ripe vanilla bean containing ca. 80% water into a cured vanilla pod that is aromatic, dark brown and which contains a water content of 20–30%. The process involves elements of biochemistry, chemistry, wound response, plant senescence and plant microstructure/compartmentation, and as such requires an integrated approach.[3–6] In general terms, the major biochemical activities are initiated during the sweating stage. Following the major loss of tissue integrity associated with the senescence process, there is activation of oxido-reductases as well as an increase in hydrolytic reactions. The consequences of these include hydrolysis of phenolic glucosides and subsequent oxidation of the liberated phenols by peroxidase and/or polyphenoloxidase. These same and other oxidative enzymes may also be involved in transformation of polyunsaturated membrane lipids by direct oxidation or indirectly by coupled reactions. The transformations occurring during sunning/night storage are less well understood. It is likely, however, that as a consequence of the relatively high moisture content and the retained activity of the endogenous peroxidase, there remains residual enzymatic catalytic capability.[7] At some stage during the curing process—and probably during the later part of the sunning/boxed-at-night phase—the enzymatic activity declines; the process is dominated by chemical and other transformations of precursors generated during fermentation. These precursors can include amino acids, reducing sugars, oxidized polyunsaturated lipids and phenols in various stages of oxidation. The nature of these reactions can only be the subject of speculation at this juncture. A similar situation exists for the drying and conditioning stages of curing, with little detailed established information available on the reactions involved in relationship to flavor generation. These areas merit further research activity.

Vanilla Aroma

Ripe green vanilla beans have no significant aroma. Volatile compounds are only generated in the vanilla bean during curing, initially in response to wounding (blanching, mechanical cutting or crushing) and later during the accelerated senescence process associated with the sweating and sun drying/boxed-overnight storage. The former is manifest by the transient appearance of the short chain C_6 unsaturated and saturated aldehydes, namely *cis*-3-hexenal and hexanal, respectively, derived via the classical oxylipin pathway, and using the substrates α-linolenic and linoleic acids. These reactions are catalyzed sequentially by the enzymes galactolipase, lipoxygenase and hydroperoxide lyase.[8] Damage to plant tissues also results in the transient appearance of the odor active compound indole. In the case of mechanically damaged and fermenting vanilla beans, this nitrogen heterocyclic compound reaches a maximum level between 1 and 3 hours after initiation of the fermentation process.[3] Indole is an intermediate in the final stage of the primary metabolic pathway leading from indole-3-glycerophosphate, catalyzed by tryptophan synthase to the amino acid tryptophan. Mechanical damage of tissues, elicited by herbivores, activates a transient secondary metabolic pathway, via indole-3-glycerophosphate lyase, liberating free indole.[9] Indole in many plants serves as a component of a volatile cocktail that signals the natural enemies of feeding herbivores.[10] The above compounds may have a role to play in plant defense.

The vast majority of the flavor volatiles of vanilla beans arise during the fermentation, sunning, drying and conditioning stages of the curing process. The flavor of vanilla extracts is complex—more than 250 compounds have been identified in cured beans, with the composition dependent on the vanilla bean's genetic background, geographical origin, local method of curing, the extraction process and the solvents employed. Recent work has simplified our understanding of the key organoleptically important aroma molecules. Using gas chromatography-odor assessment of extracts of traditionally cured Mexican vanilla beans, 24 aroma active compounds were

highlighted, of which 13 were phenolic in nature. Two C_4 α-dioxy compounds and five short chain carboxylic acids—including straight chain even and odd number acids and branched chain equivalents—were also important aroma contributors. Aldehydes were represented by a C_7 and C_{10} monounsaturated and two diunsaturated C_{10} compounds.[11] The potential biochemical/chemical origins of these aroma compounds have been examined elsewhere and will not be considered in this review.[3,12]

Vanilla Taste and Mouthfeel Compounds

A typical vanilla extract from cured beans presents a complex matrix known to contain, besides the aroma active molecules described above, a range of non-volatile compounds that include the following:[13]

Carbohydrates 25% (glucose, mannose, rhamnose)
Cellulose 15%
Fat 15%
Minerals 6%
Lignin 2-4%
Vanillin 2%
Proteins 0.5%
Water 10-35%

There is a paucity of knowledge on the taste and mouthfeel components from cured vanilla beans; this contrasts with the extensive information on aroma compounds in the same product. Little information is available on the composition of the fat fraction of the green or cured vanilla beans, i.e. content of mono-, di- or tri-glycerides and other complex lipids, though it is anticipated that the vanilla pod during development and at maturity and having photosynthetic capacity should contain polyunsaturated fatty acids high in linoleic and α-linolenic acids esterified to monogalactosyldiacylglycerol and digalactosyldiacylglycerol as complex lipids.[14]

Significant quantities of a novel lipid product family of β-dicarbonyl compounds were identified in both ripe and cured vanilla beans. These compounds were long chain aliphatics with a terminal 2,4-dicarbonyl function and a *cis* double bond at the *n*-9 position. They represent ca. 28% of the neutral lipids—that is, 1.5% in immature beans, and 10% of the neutral lipids, i.e. 0.9%, of the mature beans. A total of five β-dicarbonyl compounds have been identified, including 16-pentacosene-2,4-dione, 18-heptacosene-2,4-dione, 20-nonacosene-2,4-dione, 22-hentriacontene-2,4-dione and 24-tritriacontene-2,4-dione. The major constituent 18-heptacosene-2,4-dione (nervonoylacetone) represented 74.5% of the β-dicarbonyl fraction.[15]

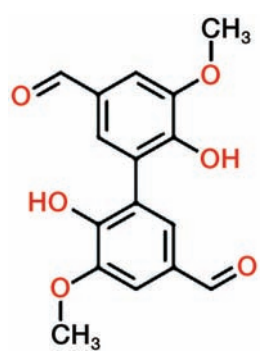
Green and cured vanilla beans also contained, in the neutral lipid fraction before saponification, a new product family in this genus, namely long-chain γ-pyrone compounds with an aliphatic chain containing a *cis* double bond at the *n*-9 position. These compounds represent 7–8% of the neutral lipids in mature beans. Three γ-pyrones have been identified, including 2-(10-nonadecenyl)-2,3-dihydro-6-methyl-4*H*-pyran-4-one, 2-(12-heneicosenyl)-2,3-dihydro-6-methyl-4*H*-pyran-4-one and 2-(14-tricosenyl)-2,3-dihydro-6-methyl-4*H*-pyran-4-one. The major constituent was 2-(14-tricosenyl)-2,3-dihydro-6-methyl-4*H*-pyran-4-one, which represented 70.3% of the γ-pyrone fraction. This compound family has been found in *V. fragrans* and *V. tahitensis* beans, but not found either in leaves or stems from the same genus or in *V. madagascariensis* beans.[16]

The function of these lipids in the vanilla bean is not known, though they could be involved in limiting water evaporation from the epidermal surface of the bean. Their function in cured vanilla bean extracts is also not established. Their biosynthetic pathways again are not defined, but they are likely to be derived by chain extension of typical C_{18} fatty acids present in the vanilla bean, with further functionalization to generate the β-dicarbonyl or γ-pyrone groups. They may be precursors of shorter chain aldehydes, the latter being derived by lipid oxidation processes, as indicated above, and may in fact contribute a mouthfeel function *per se* in cured vanilla extracts. As such, they represent significant families of non-phenolic non-volatile compounds.

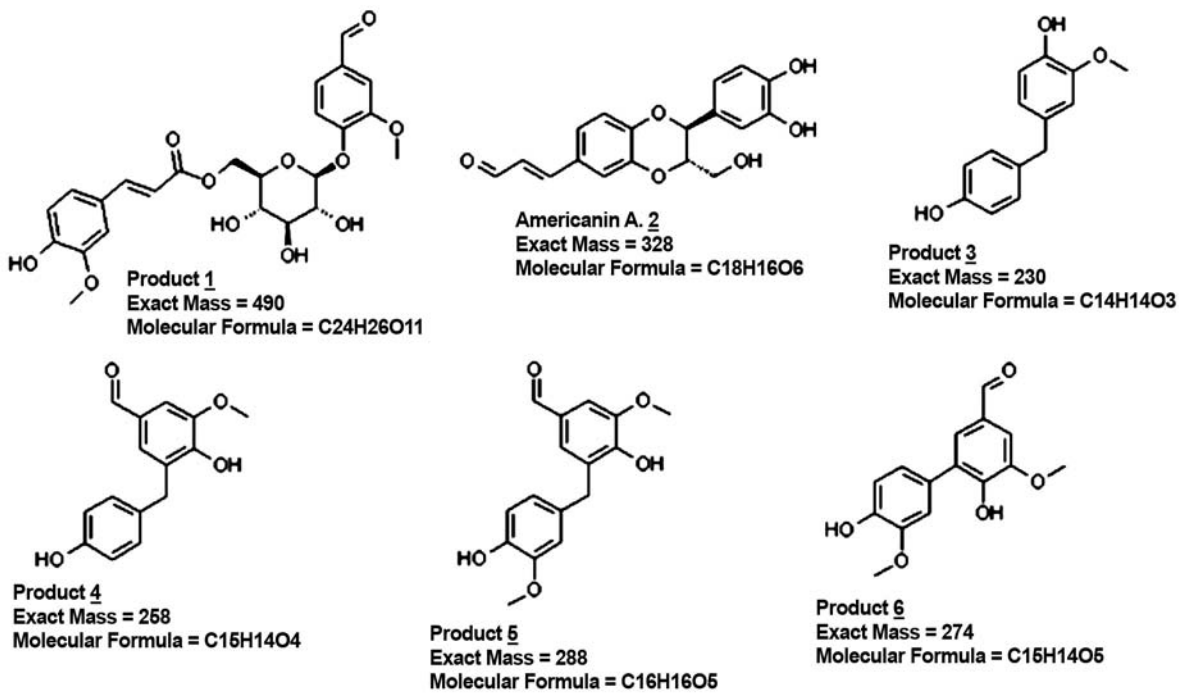
Interest in non-volatile derivatives of vanilla-type phenolics was first reported in 1915 when the dimer dehydro-divanillin (**see F-1**) was synthesized by chemical oxidation of vanillin, then much later via horseradish peroxidase/hydrogen peroxide oxidation of the same molecule.[17,18]

Divanillin and a family of unidentified vanillin oligomers were reported in cured vanilla beans of Madagascan origin.[19] This dehydro-dimer was found at a level of 170 ppm in ethanol/water extracts of Madagascan cured vanilla beans. It was proposed that the compound was formed in the vanilla bean by the action of peroxidase/hydrogen peroxide on vanillin. Divanillin was shown to have novel sensory properties. At 5–50 ppm it positively influenced the creamy, fatty properties of selected flavors and the mouthfeel and fullness of foodstuffs.[19] In-depth sensory analysis by the same workers confirmed these interesting properties. Typical applications for divanillin were in flavors developed for reduced-fat versions of yogurt, ice cream, ultra-high temperature treated milk and instant desserts. [20]

Structure of divanillin **F-1**



Structure of velvety mouth-coating compounds isolated from cured Madagascan vanilla beans[21] **F-2**



Schwarz and Hofmann significantly extended the general knowledge of vanilla taste and mouthfeel compounds by identification, from traditionally cured vanilla beans, of a number of mouthfeel compounds, in addition to the already reported divanillin.[21] The approach taken was to systematically locate the most intense taste-active compounds by application of taste dilution analysis on ethanol/water extracts of traditionally cured Madagascan vanilla beans.[22] Taste dilution factors (TDF)—the dilution at which a difference was still detected relative to a blank—were determined by the half-tongue test.[23,24]

The initial stage of the fractionation procedure was conducted on ethanol/water extract of vanilla beans after first removing the extracting solvents. The solvent-free extract was taken up in water and sequentially extracted with pentane, then ethyl acetate, leaving a final aqueous solution. After removal of all solvents the pentane, ethyl acetate and aqueous residue were recovered and yielded, respectively, 2%, 10.1% and 87.9% by weight. The pentane fraction essentially contained the volatile aroma compounds. The pentane, ethyl acetate fractions and the aqueous residue were evaluated in water in their extraction ratios by a sensory panel. The taste qualities of these fractions were then judged by an expert panel on a scale of 0 (not detectable) to 5 (strong impression).

A "velvety mouth-coating taste sensation" was shown to be very strong in the ethyl acetate fraction (4), weaker in the pentane fraction (2) and weaker still in the aqueous residue (1). Sensory panel training for recognition of the velvety mouth-coating character was carried out using quercetin-3-*O*-β-D-glucopyranoside (0.01 mmol/L). Furthermore, the results showed that a slightly sour taste seems to be located in the aqueous residue (1.0) and ethyl acetate fraction (0.5). It is interesting to note that about 88% of a vanilla extract appears to have little significant contribution to the overall flavor of the cured bean extract. Indeed, it poses the question as to the functional role of virtually 88% of the total extractable material from cured vanilla beans. Based on the above results, the ethyl acetate

Recognition thresholds of velvety mouth-coating products isolated from solvent extracts of cured Madagascan vanilla beans[21] **T-1**

Compound (#)	TCR[a]
vanillin	250
vanillin-*O*-β-D-glucopyranoside	25
5-(4-hydroxybenzyl)vanillin (4)	5.0
americanin A (2)	4.0
4-(4-hydroxybenzyl)-2-methoxyphenol (3)	3.0
4-hydroxy-3-(4-hydroxy-3-methoxybenzyl)-5-methoxybenzaldehyde (5)	2.0
(1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glucopyranoside (1)	1.5
divanillin	1.0
4′, 6′-dihydroxy-3′, 5-dimethoxy-[1, 1′-biphenyl]-3-carboxaldehyde (6)	1.0

[a]Recognition threshold concentrations, μmol/L, in water by means of the half-tongue test

Concentration and concentration/taste threshold values (DoT) of selected velvety mouth-coating products in green and cured vanilla beans **T-2**

Compound	Concentration[a] (DoT)[b]		Taste threshold[c]
	PNG[d] ripe	PNG cured	
(1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glucopyranoside (1)	<0.1 (<1)	20.2 (135)	0.15
divanillin	n.d (<1)	32.8 (328)	0.10
4', 6'-dihydroxy-3', 5-dimethoxy-[1, 1'-biphenyl]-3-carboxaldehyde (6)	n.d (<1)	37.5 (375)	0.10
americanin A (2)	16.6 (42)	64.6 (161)	0.40

[a]µmol/100g dry weight; [b]concentration/threshold; [c]µmol/100g water; [d]Papua New Guinea beans (*Vanilla planifolia*)

fraction was used as the starting point for the chemical characterization of the velvety mouth-coating compounds.

Isolation of the purified species was achieved by liquid chromatography-mass spectrometry (LC-MS) and chemical structures determined by ^{2}H- and ^{13}C-NMR. These means were used to isolate and identify six velvety mouth-coating phenolic compounds in addition to the previously isolated divanillin. The compounds, coded products 1 to 6 (**see F-2**), had not previously been reported in the literature as velvety mouth-coating tastants in vanilla; of these products, 1, 4, 5 and 6 had not previously been reported in the literature.

Product 1 was chemically related to glucovanillin, the precursor of vanillin in ripe green beans, but contained an additional molecule of ferulic acid esterified via its carboxyl group to the 6 position of gluco-D-pyranose. The dimer, americanin A (product 2), was previously identified in American pokeweed, *Phytolacca americana*.[25] Products 3, 4 and 5 were methylene biphenyls. Product 6 exhibited a biphenyl structure similar to that of divanillin.

Human recognition threshold concentrations—µmol/L in water—of vanillin, divanillin, vanillin-glucopyranoside and the six velvety mouth-coating products were determined by the half-tongue test (**see T-1**).

It can be seen from the above data that compared to vanillin all of the other compounds exhibited generally much lower velvety mouth-coating thresholds. Dimerization, as in products 2–6, showed thresholds in the range of 1–5 µm/L in water. The lowest threshold values determined were for divanillin and the biphenyl compound 4', 6'-dihydroxy-3', 5-dimethoxy-[1, 1'-biphenyl]-3-carboxaldehyde. It is interesting to compare the thresholds of vanillin (250), vanillin-*O*-β-D-glucopyranoside (25) and (1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glucopyranoside (1.5). As the phenol monomer is extended by addition of a glucose unit the threshold decreases by a factor of 10. As a further residue is added, namely ferulate, the threshold decreases to a factor of 166 relative to vanillin. A similar situation exists for the monomeric vanillin compared to the dimeric structures where the thresholds range from 50 to 250 times lower than vanillin.

Concentration and concentration/taste threshold values (DoT) of selected velvety mouth-coating compounds in green and cured vanilla beans is shown in **T-2**.

From T-2 it is clear that products 1 and 6 and divanillin were present only in cured vanilla beans. The same situation applied for products 3, 4 and 5 (data not shown). Americanin A, on the other hand, was present in the green bean but was enhanced during curing. This suggests that all of the compounds, including divanillin, are produced from ripe green bean precursors during the curing process. The fermentation stage is a potential candidate for these transformations

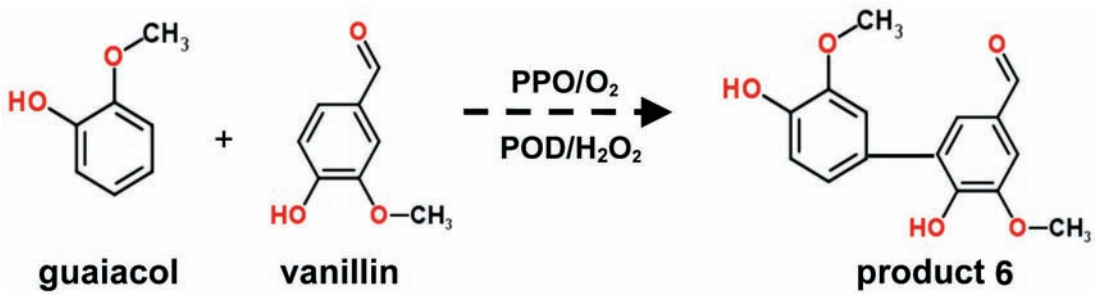
It is well established that diphenol-linked compounds can be produced by phenol oxidation reactions mediated by chemical oxidizing agents, or enzymatically via horseradish peroxidase/hydrogen peroxide.[17,18] The principal oxidoreductases reported in green vanilla beans with phenols as substrates are peroxidase and polyphenoloxidase, and perhaps laccase.[26,27]

As previously indicated, there are three categories of compounds among the velvety mouth-coating compounds isolated from cured vanilla beans, namely the phenol dimers of which there are two groups—the carbon bridged biphenyls (e.g. product 6) and the methylene biphenyls (products 3, 4 and 5), and secondly the *O*-7-8 linked lignans exemplified by americanin A. The third group is the single phenol-monosaccharide-ferulate compound, which is chemically related to vanillin-β-D-glucopyranoside. The mechanism of the formation of these families of compounds has not been established, but it is clearly related to the curing process.

The dibenzylbutyrolactone lignan americanin A is probably formed by peroxidase/polyphenoxidase catalyzed free radical dimerization of two caffeic acid residues, one of which contains an aldehyde function at the terminal position of the C_3 side chain of this phenylpropanoid. Linkage between the two monomers is via the 7-8 double bond of one C6-3 unit to the two *o*-dihydroxyl groups of the second caffeic acid residue.[28]

In the case of (1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glucopyranoside, the ferulic acid residue may provide a link to larger precursor structures. Ferulic acid and dehydrodiferulic acids are important components of sugar-beet pectins and maize bran heteroxylans.[29] In sugar-beet, ferulic acid is ester-linked mainly to the O-2 of arabinose residues and to the O-6 of galactose residues in the pectin side chain. In addition the dimer, dehydrodiferulic acid, linked by 8-5', 5-5', 8-8' and 8-O-4' bridges, is present and suggests that covalent cross-linking of pectic polysaccharides may occur through diferulate bridges in sugar-beet pulp. In the side chain of heteroxylans

Potential enzymatic routes to the biphenyl (product 6) **F-3**



of maize bran ferulic acid is esterified to the O-5 of arabinofuranose residues. In addition, 8-8', 8-5', 8-O-4' and 5-5' diferulate residues were detected. 1-O-Vanilloyl-(6-O-feruloyl)-*ß*-D-glucopyranoside, as indicated above, is absent in the ripe green vanilla bean, but present after the curing process. Based on this observation it is reasonable to conclude that the phenolic hydroxyl group on the ferulate residue of product 1 could be linked to another ferulate residue as a diferulate unit, or via the phenolic hydroxyl group to a -COOH group on a pectin backbone. In the latter case, ester hydrolysis could liberate 1-*O*-vanilloyl-(6-*O*-feruloyl)-ß-D-glucopyranoside from such a linkage. Alternatively, the -CHO group on the vanillin residue could link, via Schiff's base formation, to an available -NH_2 group of protein-linked amino acids (e.g the ε-amino group of lysine). It is of interest to note that there may be an interrelationship among the precursor(s) of 1-*O*-vanilloyl-(6-*O*-feruloyl)-ß-D-glucopyranoside, the compound itself, vanillin-*O*-ß-D-glucopyranoside and vanillin, both from biosynthetic and degradative pathway considerations.

The origin of the biphenyl compound, product 6, and divanillin could both be via similar pathways. Divanillin has already been shown to be formed from vanillin in the presence of horseradish peroxidase and hydrogen peroxide.[18] In the case of compound 6, the likely precursors are guaiacol and vanillin. Free radical coupling via the *para* position of the former to the *ortho* position of the latter could realize the appropriate dehydrodimer. The catalysts for this reaction could be the endogenous peroxidase (POD) in the presence of hydrogen peroxide (H_2O_2) and/or the vanilla bean polyphenoloxidase (PPO) using molecular oxygen (O_2) as the hydrogen acceptors (**F-3**).

The methylene biphenyls—products 3, 4 and 5—could arise in a similar way, with the methylene group derived from, e.g., the methylene carbon radical of vanillyl alcohol. If this radical reacted with the carbon-based radical at the aromatic ring *ortho* position of vanillin, then product 5 could be formed. There may be alternative, non-radical routes to this methylene biphenyl family by covalent interactions between the appropriate starting phenols; however, this is speculation at this stage. Utilization of the isolated peroxidase(s) and polyphenoloxidase(s) from green vanilla beans could provide the catalysts to test the above suggestions.[30]

Based on the recent work carried out in the area of cured vanilla bean flavor it is clear that there exists:

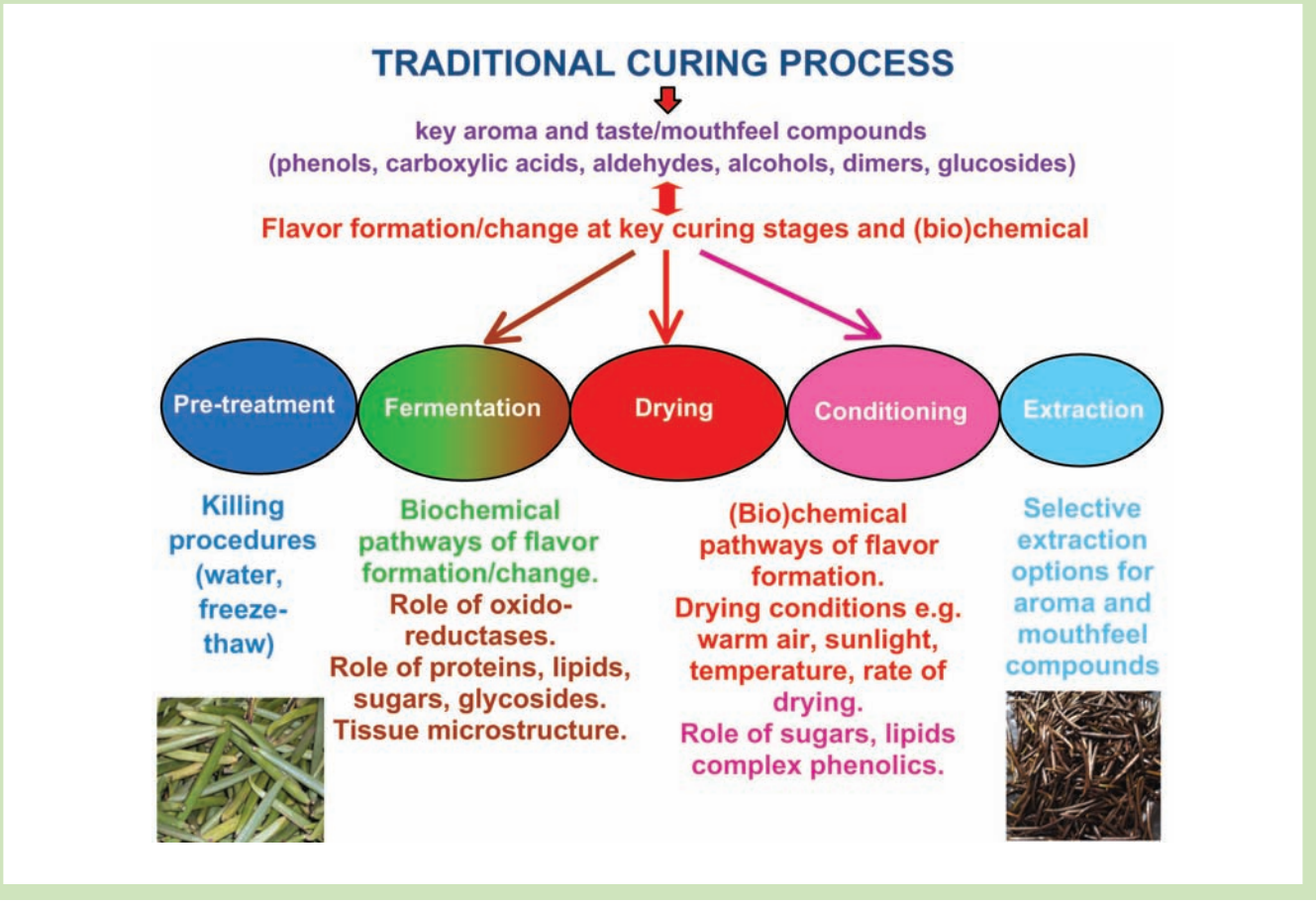
- A good knowledge of many/most of the important aroma compounds. Additional information on *Vanilla planifolia* will probably come from the same genotype grown in different geographical locations. The globally lesser crop of the related species, *Vanilla tahitensis*, on the other hand, being of different genetic origin, is known to contain mostly unique aroma components, namely anisyl alcohol, anisic acid and *para*-anisaldehyde, though with many compounds in common with *V. planifolia*.[31]
- A developing picture of the non-volatile, taste/mouthfeel compounds. The identification of the novel families of dimeric phenols, including the already known divanillin, and the more complex ferulic ester derivatives opens up new avenues in vanilla flavor. It remains to be seen what additional compounds will be found and their precursors and pathways of formation during the curing process. Similar remarks apply to the long-chain β-dicarbonyls and the γ-pyrones both with respect to their biogenetic origin and their functional role in cured vanilla bean extracts.

One of the major goals of vanilla curing is to develop a fuller understanding of vanilla flavor in terms of the key compound drivers for aroma, taste and mouthfeel. With such knowledge the determination of the biochemical and chemical pathways leading to the formation of the key flavor compounds represents a realistic focused objective (see **F-4**).

Information on the flavor component pathways can be overlaid on the different phases of the curing process to highlight the specific curing stages responsible for the formation of particular families of flavor compounds such as the monomeric and the dimeric phenols. The final stage would involve interventions in the curing process to optimize key flavor drivers.

In the future, the traditional curing process could eventually become just one facet of a series of biochemical

Key flavor formation during the vanilla bean curing stages **F-4**



and chemical interventions directed towards a fuller flavor development, or optimization of fingerprint flavor directions such as aroma or taste/mouthfeel. To realize this final goal, one needs a greater understanding of flavor formation/transformation; biochemistry/chemistry, particularly the sweating/fermentation stage of curing; as well as the not so well understood role of sunning/drying and conditioning. With these approaches it may be possible to realize the conversion of some of the 88% of the current "inert" material extracted from the cured vanilla beans into aroma, taste or mouthfeel active materials.

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References

1. SK Bahaatacharjee and KN Shiva, In: *Vanilla: The World's most Flavored Spice Orchid of Commerce*, 1–3, Aavishkar Publishers, Jaipur, India (2008)
2. SK Bahaatacharjee and KN Shiva, In: *Vanilla: The World's most Flavored Spice Orchid of Commerce*, 104–110, Aavishkar Publishers, Jaipur, India (2008)
3. P Dunphy and K Bala, Vanilla Curing: The Senescent Decline of a Vanilla Bean, *Perfum Flavor*, 34, 34–40 (2009)
4. K Hammond-Kosack and JDG Jones, In: *Biochemistry and Molecular Biology of Plants*, Eds, B Buchanan, W Gruissem and R Jones, 1102–1156, American Society of Plant Physiology, Rockville, MD (2000)
5. LA Del Rio, GM Pastori, JM Palma, IM Sandalio, F Sevilla, FJ Corpus, A Jiminez, E Lopez-Heurtas and J Hernandez, Natural Senescence of Pea Leaves (An Activated Oxygen Mediated Function for Peroxisomes), *Plant Physiol*, 116, 1195–1200 (1998)
6. E Odoux, J Escoute, JL Verdeil and JM Brillouet, Localisation of β-Glucosidase Activity and Glucovanillin in Vanilla Beans, *Ann Bot*, 92, 437–444 (2003)
7. MJW Dignum and R Verporte, Enzymes Involved in Vanilla Curing, In: *Vanilla: 1st International Congress*. 79–87, Center for Flavor Education/Allured Publishing Corp, Carol Stream, IL (2005)
8. E Blee, Phytooxylipins and Plant Defence Reactions, *Prog Lipid Res*, 37, 33–72 (1998)
9. M Frey, C Stettner, PW Pare, EA Schmelz, H Tumlinson and A Gierl, A Herbivore Elicitor Activates the Gene for Indole Emission in Maize, *PNAS*, 97, 14801–14806 (2000)

10. TCJ Turlings, JH Tumlinson and WJ Lewis, Exploitation of Herbivore Induced Odors by Host-Seeking Parasitic Wasps, *Science*, 250, 1251–1253 (1990)

11. A Perez-Silva, E Odoux, P Brat, F Ribeyre, G Rodriguez-Jiminez, V Roblez-Olvera, MA Garcia-Alvarado and Z Gunata, GC-MS and GC-Olfactometry Analysis of Aroma Compounds in a Representative Organic Aroma Extract from Cured Vanilla Beans (*Vanilla planifolia* G. Jackson), *Food Chem*, 99, 728–735 (2006)

12. P Dunphy, *Vanilla Review: It's all a matter of Taste*, Presented at Vanilla 2009—Vanilla Diseases: Past History and Future Prospects. Jamesburg, NJ, USA, Nov 10–11 2009

13. SR Rao and GA Ravishankar, Vanilla Flavor: Production by Conventional and Biotechnological Routes, *J Sci Food Agric*, 80, 289–304 (2000)

14. C Somerville, J Browse, JG Jaworski and JB Ohlrogge, In: *Biochemistry and Molecular Biology of Plants*, Eds, B Buchanan, W Gruissem and R Jones, American Society of Plant Physiology, Rockville, MD, 456–527 (2000)

15. B Ramaroson-Raonizafinimanana, M Emile, EM Gaydou and I Bombarda, Long-Chain Aliphatic β-Diketones from Epicuticular Wax of *Vanilla* Bean Species. Synthesis of Nervonoylacetone, *J Agric Food Chem*, 48, 4739–4743 (2000)

16. B Ramaroson-Raonizafinimanana, M Emile, EM Gaydou and I Bombarda, Long-Chain γ-Pyrones in Epicuticular Wax of Two Vanilla Bean Species: *V fragrans* and *V tahitensis*, *J Agric Food Chem*, 47, 3202–3205 (1999)

17. K Elbs and H Lerch, Uber Dehydrovannillin, *J Prakt Chemie*, 2, 1–9 (1915)

18. J Baumgartner and H Neukom, Enzymatische Oxydation von Vanillin, *Chimia*, 26, 366–368 (1972)

19. I Gatfield, I Reis, G Krammer, CO Schmidt, G Kindel and HJ Bertram, Novel Taste-Active Components of Fermented Vanilla Beans, *Perfum Flavor*, 31, 18–20 (2006)

20. I Reiss, IL Gatfield, G Krammer, A Clere and G Kindel, US Patent Application Publication, *Pub No US 2006/0286237 A1*. Dec 21 (2006)

21. B Schwarz and T Hofmann, Identification of Novel Orosensory Active Molecules in Cured Vanilla Beans (*Vanilla planifolia*), *J Agric Food Chem*, 57, 3729–3737 (2009)

22. O Frank, H Ottinger and T Hofmann. Characterisation of an Intense Bitter-Tasting *1H,4H*-Quinolizinium-7-olate by Application of the Taste Dilution Aanalysis, a Novel Bioassay for the Screening and Identification of Taste Active Compounds in Foods, *J Agric Food Chem*, 49, 1336–1344 (2001)

23. S Scharbert, N Holzmann and T Hofmann. Identification of the Astringent Taste Compounds in Black Tea Infusions by Combining Instrumental Analysis with Human Bioresponse, *J Agric Food Chem*, 52, 3498–3508 (2004)

24. A Brock and T Hofmann, Identification of Key astringent Compounds in Spinach (*Spinacia oleracea*) by Means of Taste Dilution Analysis, *Chem Percept*, 1, 268–281 (2008)

25. M Shoeb, SM MacManus, Y Kumarasamy, M Jaspers, L Nahar, PK Thoo-Lin, H Nazemiyeh and SD Sarker, Americanin a: A Biactive Dibenzyl Butyrlactone from the Seeds of *Centaurea americana*, *Phytochem*, 67, 2370–2375 (2006)

26. MA Ferrer, M Gomez-Tena, MA Pedreno and A Ros-Barcelo, Postharvest Effect of Ethrel on Peroxidase of Iceberg Lettuce Leaf Tissue, *Biol Technol*, 7, 301–307 (1996)

27. D Havkin-Frenkel, J French, F Pak and C Frenkel, Inside Vanilla: *Vanilla planifolia's* Botany, Curing Options and Future Marketing Prospects, *Perfum Flavor*, 30, 36–55 (2005)

28. R Croteau, TM Kutchan and NG Lewis, In: *Biochemistry & Molecular Biology of Plants*, Eds, B Buchanan, W Gruissem and R Jones, R Am Soc Plant Physiol, 1250–1318 (2000)

29. L Saulnier and J-F Thibault, Ferulic Acid and Diferulic Acids as Components of Sugar-Beet Pectins and Maize Bran Heteroxylans, *J Sci Food Agric*, 79, 396–402 (1999)

30. O Marquez, KN Waliszewski, RM Oliart and VT Pardio, Purification and Characterisation of Cell Wall-Bound Peroxidase from Vanilla Beans, *Food Sci Technol*, 41, 1372–1379 (2008)

31. SK Bahaatacharjee and KN Shiva, In: *Vanilla: The World's most Flavored Spice Orchid of Commerce*, Aavishkar Publishers, Jaipur, India, 111–119 (2008)